AMENDMENTS TO THE CLAIMS

The below listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Original) A method for modulating endothelial cell (EC) proliferation in a mammal, wherein the method comprises increasing or decreasing ezrin activity in the mammal by an amount sufficient to modulate proliferation of the cells.

2. (Original) The method of claim 1, wherein the ezrin activity is decreased by an amount sufficient to enhance the EC proliferation.

3. (Previously presented) The method of claim 1, wherein the method further comprises administering to the mammal at or near a site where modulation is desired at least one ezrin modulating agent sufficient to decrease the ezrin activity and enhance the EC proliferation.

4. (Withdrawn) The method of claim 3, wherein the ezrin modulating agent is a nucleic acid or at least one amino acid sequence.

5. (Withdrawn) The method of claim 4, wherein the amino acid sequence is a competitor of Tumor Necrosis Factor-alpha (TNF-α).

6. (Withdrawn) The method of claim 5, wherein the competitor is at least one of TNF soluble receptor protein (TNFsr), a TNF antagonist, anti-TNF antibody; or an effective fragment or derivative thereof.

7. (Withdrawn) The method of claim 4, wherein the ezrin modulating agent is an anti-sense nucleic acid, anti-ezrin antibody, or an effective fragment or derivative thereof.

8. (Original) The method of claim 3, wherein the ezrin modulating agent reduces or blocks activity of Rho kinase (ROCK-2) in the endothelial cells.

9. (Original) The method of claim 8, wherein the ezrin modulating agent is Y27632.

10. (Withdrawn) The method of claim 4, wherein the ezrin modulating agent is a nucleic acid encoding a dominantly and negatively acting fragment of mammalian ezrin; or an effective fragment or derivative of the protein.

11. (Original) The method of claim 1, wherein the decrease in ezrin activity is at least about 50% as determined by a standard cyclin A promoter binding assay.

12. (Original) The method of claim 1, wherein the decrease in ezrin activity is at least about 50% as determined by a standard ezrin mRNA stability assay.

13. (Original) The method of claim 2, wherein the decrease in ezrin activity is associated with a decrease in ezrin tyrosine phosphorylation as determined by a standard protein phosphorylation assay.

14. (Withdrawn) The method of claim 1, wherein the ezrin activity is increased in an amount sufficient to decrease the EC proliferation.

15. (Withdrawn) The method of claim 14, wherein the ezrin modulating agent is tumor necrosis factor (TNF) or an effective fragment thereof.

16. (Withdrawn) The method of claim 14, wherein the EC proliferation is decreased by at least about 20% as determined by a standard restenosis assay.

17. (Previously presented) A method for inducing formation of new blood vessels in a mammal, the method comprising decreasing ezrin activity in an amount sufficient to induce formation of the new blood vessels in the mammal.

18. (Original) The method of claim 17, wherein the method further comprises administering to the mammal at least one ezrin modulating agent sufficient to decrease ezrin DNA binding activity relative to a control.

19. (Original) The method of claim 18, wherein the ezrin modulating agent is an inhibitor of Rho kinase (ROCK-2).

20. (Withdrawn) The method of claim 18, wherein the ezrin modulating agent is a nucleic acid encoding a dominantly and negatively acting fragment of mammalian ezrin; or an effective fragment or derivative of thereof.

21. (Previously presented) The method of claim 18, wherein the method further comprises contacting endothelial cells (ECs) with the ezrin modulating agent ~~sufficient to decrease~~ thereby decreasing ezrin activity in the cells.

22. (Withdrawn) The method of claim 21, wherein the method further comprises transforming endothelial cells with the ezrin modulating agent under conditions conducive to expressing the agent and administering the transformed cells to the mammal.

23. (Withdrawn) The method of claim 22, wherein the ezrin modulating agent is a nucleic acid encoding a dominantly and negatively acting fragment of mammalian ezrin; or an effective fragment or derivative thereof.

24. (Original) The method of claim 17, wherein the mammal has, is suspected of having, or will have ischemic tissue.

25. (Original) The method of claim 24, wherein the tissue is associated with an ischemic vascular disease.

26. (Previously presented) The method of claim 1 or 17, wherein the method further comprises administering to the mammal at least one of an angiogenic protein, cytokine, hematopoietic protein , or an effective fragment thereof.

27. (Previously presented) The method of claim 26, wherein the angiogenic protein is acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF-1), epidermal growth factor (EGF), transforming growth factor α and β (TGF-α and TFG-β), platelet-derived endothelial growth factor (PD-ECGF), platelet-derived growth factor (PDGF), tumor necrosis factor α (TNF-α), hepatocyte growth factor (HGF), insulin like growth factor (IGF), erythropoietin, colony stimulating factor (CSF), macrophage-CSF (M-CSF), angiopoetin-1 (Ang1) or nitric oxide_synthase (NOS).

28. (Withdrawn) The method of claim 26, wherein the hematopoietic factor is granulocyte-macrophage colony-stimulating factor (GM-CSF), VEGF, Steel factor (SLF, also known as Stem cell factor (SCF)), stromal cell-derived factor (SDF-1), granulocyte-colony stimulating factor (G-CSF), HGF, Angiopoietin-1, Angiopoietin-2, M-CSF, b-FGF, and FLT-3 ligand.

29. (Withdrawn) The method of claim 28, wherein the protein is one of VEGF-B, VEGF-C, VEGF-2, VEGF-3; or an effective fragment thereof.

30. (Previously presented) A method for reducing the severity of blood vessel damage in a mammal, wherein the method comprises decreasing ezrin activity in endothelial cells (EC) before, during or after the mammal is exposed to conditions conducive to damaging the blood vessels , wherein the decrease in ezrin activity is sufficient to reduce the severity of the blood vessel damage in the mammal.

31. (Previously presented) The method of claim 30, wherein the method further comprises administering to the mammal at least one ezrin modulating agent sufficient to decrease ezrin DNA binding activity relative to a control.

32. (Original) The method of claim 31, wherein the ezrin modulating agent is injected at or near the site of blood vessel damage in the mammal.

33. (Original) The method of claim 32, wherein the ezrin modulating agent is an inhibitor of Rho kinase (ROCK-2).

34. (Original) The method of claim 33, wherein the ezrin modulating agent is Y27632.

35. (Withdrawn) The method of claim 30, wherein the ezrin modulating agent is a nucleic acid encoding a dominantly and negatively acting mammalian ezrin protein; or an effective fragment or derivative of the protein.

36. (Original) The method of claim 31, wherein the blood vessel damage is restenosis associated with an invasive manipulation or associated with ischemia.

37. (Original) The method of claim 36, wherein the invasive manipulation is balloon angioplasty, or deployment of stent or catheter.

38. (Original) The method of claim 37, wherein the stent is an endovascular stent.

39. (Original) The method of claim 36, wherein the ischemia is associated with at least one of infection, trauma, graft rejection, cerebrovascular ischemia, renal ischemia, pulmonary ischemia, limb ischemia, ischemic cardiomyopathy, or myocardial ischemia.

40. (Original) The method of claim 30, wherein the ezrin modulating agent is administered to the mammal at least about 12 hours before exposing the mammal to the conditions conducive to damaging the blood vessels.

41. (Original) The method of claim 40, wherein the ezrin modulating agent is administered to the mammal between from about 1 to 10 days before exposing the mammal to the conditions conducive to damaging the blood vessels.

42. (Original) The method of claim 41, wherein the method further comprises administering the ezrin modulating agent to the mammal following the exposure to the conditions conducive to damaging the blood vessels.

43. (Withdrawn) A method for decreasing angiogenesis in a mammal, wherein the method comprises increasing ezrin activity in endothelial cells (ECs) of the mammal sufficient to decrease the angiogenesis.

44. (Withdrawn) The method of claim 43, wherein the method further comprises administering to the mammal at least one ezrin modulating agent sufficient to decrease ezrin DNA binding activity relative to a control.

45. (Withdrawn) The method of claim 44, wherein the ezrin modulating agent is injected at or near a site in which the decrease in angiogenesis is desired.

46. (Withdrawn) The method of claim 44, wherein the ezrin modulating agent is TNF Necrosis Factor alpha (TNF-α), Rho kinase; or an effective fragment or derivative thereof.

47. (Withdrawn) The method of claim 44, wherein the mammal has, is suspected of having, or is pre-disposed to develop cancer.

48. (Withdrawn) The method of claim 43, wherein the method further comprises administering at least one chemotherapeutic drug to the mammal.

49. (Withdrawn) A method for detecting a compound that modulates ezrin activity in the mammal, the method comprising the steps of:

1) introducing into cells a nucleic acid comprising at least part of a mammalian cyclin A gene linked to a detectable sequence,

2) adding at least one known or candidate ezrin modulating agent to the cells,

3) culturing the cells under conditions suited to expressing the nucleic acid and detecting the sequence in the presence and absence of the compound; and

4) determining the effect of the compound on the cells.

50. (Withdrawn) The method of claim 49, wherein step (4) of the method further comprises measuring at least one of proliferation and cycling of the cells.

51. (Withdrawn) The method of claim 49, wherein the nucleic acid used in the assay comprises a region spanning – 1200 to +250 of the mammalian cyclin A gene.

52. (Withdrawn) The method of claim 51, wherein the nucleic acid comprises a region spanning –924 to +100 of the mammalian cyclin A gene.

53. (Withdrawn) The method of claim 52, wherein the nucleic acid comprises at least one of the API, ATF, and CDE-CMR promoter sites.

54. (Withdrawn) The method of claim 53, wherein the nucleic acid comprises the CDE-CMR promoter site between about –79 to about +100 of the mammalian cyclin A gene.

55. (Withdrawn) The method of claim 49, wherein the nucleic acid comprises the human cyclin A protein gene promoter spanning about positions –79 to about +100 of the gene which promoter is covalently linked in-frame to a sequence encoding a fluorescent or phosphorescent protein; or a detectable fragment thereof.

56. (Withdrawn) The method of claim 55, wherein the label is derived from a fluorescent jellyfish protein.

57. (Withdrawn) The method of claim 56, wherein the jellyfish protein is green fluorescent protein (GFP) or red fluorescent protein (RFP).

58. (Withdrawn) The method of claim 49, wherein the nucleic acid comprises the human cyclin A protein gene promoter spanning about positions –79 to about +100 of the gene which promoter is covalently linked in-frame to a sequence encoding the luciferase or beta-galactosidase enzyme; or a detectable fragment thereof.

59. (Withdrawn) A method for detecting a compound that modulates ezrin activity, the method comprising the steps of:

1) adding at least one known or candidate ezrin modulating agent to the cells,

2) culturing the cells under conditions suited to increase or decrease ezrin phosphorylation relative to a control; and

3) identifying an increase or decrease in ezrin phosphorylation relative to a suitable control as being indicative of the compound.

60. (Withdrawn) The method of claim 59, wherein step (3) of the method comprises performing an immunoassay.

61. (Withdrawn) The method of claim 60, wherein the immunoassay comprises performing a sandwich type immunoassay with an anti-phosphotyrosine antibody.

62. (Withdrawn) A method for detecting DNA binding between ezrin (or a DNA binding fragment thereof) and at least part of a mammalian cyclin A gene, the method comprising the steps of:

1) incubating at least part of a mammalian cyclin A gene with the ezrin protein or a DNA binding fragment thereof, wherein the incubation is conducted under conditions sufficient to form a specific binding pair between the cyclin A gene and the ezrin protein (or fragment),

2) adding at least one known or candidate ezrin modulating agent to the incubation medium; and

3) detecting presence of a specific binding pair between the cyclin A gene (or fragment) and the ezrin protein (or fragment) in the presence and absence of the compound, wherein a reduction or absence of the binding pair is taken to be indicative of a compound that reduces or blocks ezrin binding to the cyclin A gene.

63. (Withdrawn) The method of claim 62, wherein the cyclin A gene part is a detectably-labeled oligonucleotide comprising at least the CDE-CDR sequence.

64. (Withdrawn) The method of claim 63, wherein the detectable label is visualized by means of an automated or semi-automated fluorescence, colorimetric, or phosphorescence detection device.

65. (Withdrawn) The method of claim 63, wherein the specific binding pair is detected by performing an electrophoretic manipulation.

66. (Original) The method of claim 1, 17, or 30, wherein the method further comprises isolating endothelial progenitor cells (EPCs) from the mammal and contacting the EPCs with at least one of: an ezrin modulating agent, cytokine, angiogenic factor or hematopoetic factor.

67. (Original) The method of claim 66, wherein the method further comprises administering the EPCs to the mammal in an amount sufficient to modulate endothelial cell proliferation.

68. (Original) The method of claim 67, wherein the method further comprises administering at least one of the following to the mammal before, during of after administration of the EPCs: ezrin modulating agent, cytokine, angiogenic factor or hematopoetic factor.

69. (Withdrawn) A pharmaceutical product for inducing neovascularization in a mammal, wherein the product comprises endothelial cells, the product comprising at least one ezrin modulating agent, wherein cells formulated to be physiologically acceptable to a mammal.

70. (Withdrawn) The pharmaceutical product of claim 69, wherein the product is sterile and further comprises at least one angiogenic protein or nucleic acid encoding the protein.

71. (Withdrawn) The pharmaceutical product of claim 70, wherein the endothelial cells express the ezrin modulating agent.

72. (Withdrawn) The pharmaceutical product of claim 71, wherein the expression is transient.

73. (Withdrawn) A kit for the introduction of a endothelial cells into a mammal, the kit comprising at least one ezrin modulating agent and optionally at least one angiogenic or hematopoietic protein or nucleic acid encoding same, the kit further comprising a pharmacologically acceptable carrier solution, nucleic acid or mitogen, means for delivering the cells and directions for using the kit.

74. (Withdrawn) The kit of claim 73, wherein the means for delivering the endothelial cells is a stent, catheter or syringe.

75. (New) A method for increasing endothelial cell (EC) proliferation in a mammal, wherein the method comprises decreasing ezrin activity in the mammal by an amount sufficient to increase proliferation of the cells by administering to the mammal at or near a site where modulation is desired Y27632 sufficient to decrease the ezrin activity and enhance the EC proliferation.

76. (New) A method for reducing the severity of blood vessel damage in a mammal, wherein the method comprises decreasing ezrin activity in endothelial cells (EC) before, during or after the mammal is exposed to conditions conducive to damaging the blood vessels, wherein the decrease in ezrin activity is sufficient to reduce the severity of the blood vessel damage in the mammal by administering to the mammal Y27632 at or near the site of blood vessel damage sufficient to decrease ezrin DNA binding activity relative to a control.

REMARKS

Claims 4-7, 10, 14-16, 20, 22, 23, 28, 29, 35, 43-65, and 69-74 are withdrawn; claims 3, 17, 21, 26, 27, 30, and 31 were previously presented; claims 1-3, 8, 9, 11-13, 16-19, 21, 24-27, 30-34, 36-42, and 66-68 are pending; and claims 75 and 76 are new. Claims 1-3, 8, 9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, and 66-68 are rejected under 35 U.S.C. §112, first paragraph. Each of the rejections is addressed below.

Support for the Amendments

Support for the amendments is found throughout the specification and claims as originally filed. For example, support for new claim 75 is at least found in the claims as originally filed and in the specification as originally filed in the paragraph at page 6, lines 14 – 23. Support for new claim 76 is at least found in the claims as originally filed and in the specification as originally filed in the paragraph spanning page 6, line 25 to – page 7, line 2. No new matter has been added.

Amendment and cancellation of the claims here are not to be construed as an acquiescence to any of the rejections/objections made in the instant Office Action or in any previous Office Action, and were done solely to expedite prosecution of the application. Applicants hereby reserve the right to pursue the claims as originally filed, or substantially similar claims in one or more subsequent patent applications.

Rejections under 35 U.S.C. § 112, second paragraph, Written Description

Claims 1-3, 8, 11-13, 17-19, 21, 24-27, 30-33, 36-42, and 66-68 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The rejected claims are directed to i) methods for modulating endothelial cell (EC) proliferation in a mammal by increasing or decreasing ezrin activity (claims 1-3, 8, 11-13, and 66-68); ii) methods for inducing formation of new blood vessels in a mammal by decreasing ezrin activity (claims 17-19, 21, 24-27, and 66-68); or iii) methods for reducing the severity of blood vessel damage in a mammal by decreasing ezrin activity (claims 30-33, 36-42, and 66-68). In support of the rejection for failing to comply with the written description requirement, the Office alleges that Applicants have not provided evidence of possession of a compound that will decrease ezrin activity by

providing sufficient distinguishing identifying characteristics of the genus. For the reasons detailed below, Applicants respectfully disagree with the rejection and request that it be withdrawn.

In *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976), the court has ruled "There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed." (M.P.E.P. 2163.03). Thus, the Office has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims. Applicants respectfully submit that the Office has not provided evidence or reasons to support such a rejection according to the guidelines provided by M.P.E.P. §2163 and upheld by case law. Specifically, the court has held that

> ...in accordance with our prior case law, that (1) examples are not necessary to support the adequacy of a written description (2) the written description standard may be met (as it is here) even where actual reduction to practice of an invention is absent; and (3) there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure. *Falkner v. Inglis* 79 USPQ2d 1001 (Fed. Cir. 2006)

Thus, examples, reduction to practice, and recitation of known structure are not required to comply with the written description requirement. Rather, Applicant must merely show possession of the claimed invention. Applicant has clearly satisfied this standard.

The present claims are directed to methods for modulating endothelial cell (EC) proliferation, methods for inducing the formation of new blood vessels, or methods for reducing the severity of blood vessel damage by modulating ezrin activity. Applicants specification clearly describes the claimed inventions. For example, Applicants describe the modulation of endothelial cell proliferation and the induction of new blood vessel formation by modulating ezrin activity in Example 14. Specifically, Applicants show that disruption of ezrin activity *in vivo* increased endothelial cell proliferation and angiogenesis in a mouse hind limb ischemia model (Example 14, pages 45-46 and Figure 13). Applicants also discovered that mice that received HUVEC transfected with a mutant form of ezrin had significantly higher numbers of proliferating endothelial cells than control mice that received HUVEC transfected with wild-type ezrin. These *in vivo* results corroborate Applicants' *in vitro* data, showing that endothelial cells transfected with an ezrin protein

containing a dominant negative mutation exhibited significantly increased levels of proliferation (Example 12, pages 43-44). In view of this disclosure, one of skill in the art would appreciate that applicants were in possession of methods for modulating endothelial cell proliferation and the formation of new blood vessels by modulating ezrin activity.

In addition, Applicants have clearly described methods for reducing the severity of blood vessel damage by modulating ezrin activity. For example, Applicants have shown that reducing ezrin activity blocks TNF's suppression of endothelial cell proliferation (Examples 12 and 13, pages 43-45). Furthermore, Applicants have shown that blocking RhoA kinase activity using Y27632 reversed ezrin/TNF mediated inhibition of endothelial cell proliferation (Figure 15C and Example 16, page 47, line 25, to page 48, lines 1-11). Based on these results, one skilled in the art would expect that decreasing ezrin activity should reduce the severity of blood vessel damage when a mammal is exposed to conditions conducive to damaging the blood vessels. Again, in view of this disclosure, one of skill in the art would appreciate that Applicants were in possession of methods for reducing the severity of blood vessel damage by modulating ezrin activity.

In sum, Applicants have clearly described the claimed invention. One skilled in the art provided with Applicants' specification would clearly recognize that Applicants were in possession of the claimed invention as of the filing date. Nothing more is required. Accordingly, the written description rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph, Enablement

Claims 1-3, 8, 9, 11-13, 17-19, 21, 24-27, 30-34, 36-42, and 66-68 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The rejected claims are directed to i) methods for modulating endothelial cell (EC) proliferation in a mammal by increasing or decreasing ezrin activity (claims 1-3, 8, 9, 11-13, and 66-68); ii) methods for inducing formation of new blood vessels in a mammal by decreasing ezrin activity (claims 17-19, 21, 24-27, and 66-68); and iii) methods for reducing the severity of blood vessel damage in a mammal by decreasing ezrin activity (claims 30-34, 36-42, and 66-68). In support of the enablement rejection, the Examiner maintains

that Applicants have allegedly failed to provide *in vivo* data. In particular, at page 7, lines 20 – 21, the Examiner states:

> In the instant case, an *in vitro* experiments [sic] are not predictive of treating a mammal with a compound that decreases ezrin activity, for the reasons discussed in the previous Office Action.

For the reasons detailed below, Applicants respectfully disagree with the enablement rejection and request that it be withdrawn.

The standard set forth for enablement in 35 U.S.C. 112, first paragraph, requires that Applicants provide a description of the invention sufficient "to enable any person skilled in the art to which it pertains...to make and use" the invention. The examples provided in the specification clearly teach the methods of the claims. Specifically, the examples show that i) *In vivo* increase in endothelial cell proliferation results from reducing ezrin activity (Example 14 (pages 45-46) and Figure 13); ii) *in vivo* increase in blood vessel formation results from reducing ezrin activity (page 12, lines 1-4, Figure 13, and Example 14, page 45, line 13, to page 46, line 21); and ii) blocking RhoA kinase activity relieves ezrin/TNF mediated inhibition of endothelial cell proliferation (page 39, line 20, to page 40, line 6; Figure 15C; and Example 16, page 47, line 25, to page 48, lines 1-11). Taken together, these examples are consistent with the scope of what is claimed and would convey to one of skill in the art "how to make and use" the invention. The claims are enabled from the examples in the specification correlating *in vitro* and *in vivo* experiments. Regarding the Office's stance on enablement when such a correlation is relied upon, M.P.E.P. §2164.02 is clear that "a rigorous or an invariable exact correlation is not required," citing *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985):

> [B]ased upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, and **therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence**. (Citations omitted.) [Emphasis added]

In support of the enablement rejection the Office has once again cited Shibata et al., (*Circulation* 103:284-289, 2001; hereinafter "Shibata"). Although cited in the Office Action of

May 1, 2007, Applicants note that Uchida et al., (*Biochemical and Biophysical Research Communications* 269:633-640, 2000; hereinafter "Uchida"); and Xue et al., (*Hepatology* 499, 38 (4) Suppl 1: 400A; hereinafter "Xue") are not specifically cited in the present Office Action. Once again Applicants maintain that the question of whether or not a reference "teaches away" from Applicants' claimed method is irrelevant to the question of enablement (*Singh v. Brake*, 317 F.3d 1334 (Fed. Circ. 2002). These references which allegedly "teach away" do not provide evidence of a lack of predictability nor do they invalidate Applicants' disclosure. Responding directly to the Examiner's allegation that Shibata somehow disproves the Applicants' disclosure, Shibata's *in vivo* experiments merely teach that they were unable to detect reendothelialization by the administration of Y27632 to injured rat carotid artery compared to controls. Although they conclude that administration had no significant effects on reendothelialization following injury, this result neither supports nor detracts from Applicants' disclosure. Shibata first note that they did not assay Rho-kinase activity directly in rat carotid artery:

> First, at present, no direct method is available to assay Rho-kinase activity in the vascular samples. Also, no rescue experiment was done on the Y27632 rats. Thus, it remains possible that inhibition of other serine-threonine protein kinases in addition to Rho-kinase may have contributed to the effects of Y27632 observed in the study. Second, because Rho is not the only possible activator of Rho-kinase, it is possible that upstream pathways other than Rho would activate Rho-kinase in injured artery. (Shibata et al., at page 288, line 55 to page 289, line 4)

In the absence of this direct experiment, it is unclear from Shibata whether the administration of Y27632 decreased Rho-kinase activity and, consequently, ezrin activity. Nor does their experiment rule out the possibility that other effects may mask the effect of decreasing ezrin activity *in vivo*. In particular, Shibata discuss the possibility of other systemic effects due to their experimental design:

> Third, because Y27632 was systemically administered for a long time in the present study, the possibility of the involvement of indirect systemic effects, such as neurohumoral effects, cannot be excluded. (Shibata et al., at page 288, line 55 to page 289, line 2)

In view of these limitations, the Shibata reference is inconclusive as to whether ezrin activity is affected.

In contrast, Applicants' specification describes the action of Rho kinase inhibitors for modulating ezrin activity. As acknowledged by the Office (Office Action mailed May 1, 2007, page 6, lines 12-14), where the Examiner stated, "Applicant's *in vitro* results indicate that Y27632 could be useful *in vivo* for treating ischemic vascular disease by enhancing endothelial cell growth." Additionally, the Examiner has acknowledged at page 6, lines 9 – 11, that

> It is not unexpected that the mice receiving HUVECs transfected with a dominant negative mutation had a functionally significant increase in blood vessel formation compared to mice receiving HUVECs transfected with wild-type ezrin.

The Examiner then goes on to describe a plausible mechanism for how HUVECs with dominant-negative mutations in ezrin may convincingly work (page 6, lines 14 – 20). Guidelines for the determination of enablement based on a complete examination of the evidence is provided in M.P.E.P. §2164.05, which states:

> Once the examiner has weighed all the evidence and established a reasonable basis to question the enablement provided for the claimed invention, the burden falls on applicant to present persuasive arguments, supported by suitable proofs where necessary, that one skilled in the art would be able to make and use the claimed invention using the application as a guide. **The evidence provided by applicant need not be conclusive but merely convincing to one skilled in the art.** *In re Brandstadter*, 484 F.2d 1395, 1406-07, 179 USPQ 286, 294 (CCPA 1973) [Emphasis added].

Because the disclosures in the specification meet the standard of "convincing to one skilled in the art," the Examiner's statements carry with them a presumption of the predictability of the system, which informs one skilled in the art "how to use" the invention being claimed.

Therefore Applicants respectfully disagree with and traverse the rejection for lack of enablement corresponding to the scope of the invention. Applicants have provided *in vivo* and *in vitro* working examples that correlate with one another showing that increasing or decreasing ezrin activity is sufficient i) to modulate endothelial cell (EC) proliferation, ii) to increase angiogenesis in a mouse model of hindlimb ischemia, and ii) to reduce the severity of blood vessel damage in a mammal exposed to conditions conducive to damaging the blood vessels. For the reasons detailed above, there is no reason why the methods being claimed would not be expected to work as